BIOLOGICAL TEMPLATING AND THE PRODUCTION OF FUNCTIONAL FIBERS

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ABSTRACT

Biology offers several advantages over traditional systems for the construction of novel materials. These include self-assembly, template-directed assembly, replication, molecular diversity, and the ability to screen and select from amidst this diversity. To harness the inherent advantages of biological systems, they must be interfaced with non-biological materials. Constructing these interfaces has been difficult because electrical/mechanical/optical systems have typically not been designed to accommodate the aqueous biochemistry of living systems. Recent research studies reported from the Belcher lab (Flynn, 2003; Mao, 2004; and Nam, 2006) demonstrate that this shortcoming can be overcome by utilizing genetically controlled proteins as templates to mineralize metals and inorganic materials at room temperature. In addition, fibers can be made from genetically controlled proteins in aqueous environments (Arcidiacono, 2002). Potentially, these genetically controlled peptides can mineralize inorganic or metallic particles at the surface of these fibers. Current, manufacturing of metallic or metallic-coated fibers requires high temperature and pressure processes, which are environmentally unfriendly and costly. These biological materials could open a new synthesis route to manufacture multifunctional fibers. In this paper, we will introduce the application of a genetically controlled filamentous bacteriophage in fabrication of functional fibers. New optical and semi-conducting fibers are envisioned in addition to catalysts, energy storage and generation technologies.

1. INTRODUCTION

1.1 Mineralization and Tailorability of Inorganic and Metallic Nanoparticles

Nature's ability to control protein structure and direct the synthesis of exquisite nanostructures composed of inorganic materials has motivated the scientific community for decades. Figure 1 depicts an example of this beauty in the calcite plates and assembled sphere formed by a unicellular phytoplanktonic algae. Other examples include sea shells made from calcite and sea spine sponge from silica. Their molecular structures are nearly perfect and are synthesized at ambient temperatures, aqueous solutions and environmentally friendly. Inspired by nature, many research efforts in the last decade have focused on using organic materials to template the formation of inorganic materials (Young, 1992; Ogasawara, 2000; Flynn, 2003; Dickerson, 2004; Goede, 2004; and Orne 2005) . DNA, protein or peptide and viruses have all been explored as biological templates.

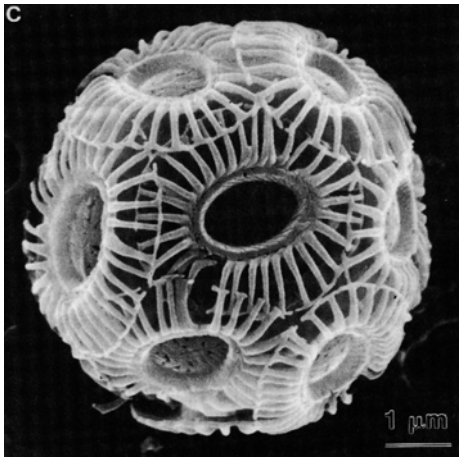

Figure 1. Micrograph of a Coccophere (protein directed synthesis of an exquisite CaCO3 structure). Stephen Mann, 1992

1.2 M13 Bacteriophage and Biopanning

M13 bacteriophage is a filamentous virus capably of infecting only bacterial cells. It is 880 nm in length and 5 to 6 nm in width. Along the virus particle are identical

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2,700 copies of pVIII proteins which encase a single strand deoxyribonucleic acid (ss-DNA). This DNA encodes all the information to replicate the DNA itself and produce assistant proteins and coat proteins for virus assembly. At one end of the virus, approximately 5 copies of functional pIII proteins are present, which bind to the bacteria host and deliver the virus DNA into the host. A transmission electron microscopic (TEM) image and a schematic structure of M13 bacteriophage are shown in Figure 2. Each type of protein in the virus capsid has its own structural conformation and functionality, and each sequence of the coat proteins can be genetically altered by modifying the viral DNA. This makes the M13 virus a manipulatable and powerful template for nano-engineering.

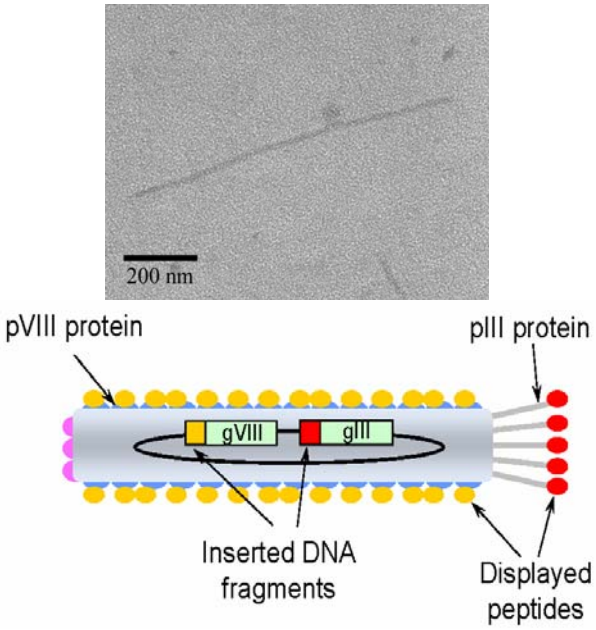

Figure 2. TEM image and schematic representation of M13 bacteriophage

The genetic engineering technique to express designed functional peptides on the virus surface is so-called phage display. It has been widely used to modify the virus surface and functionality. By using the phage display technique, short peptides containing 2 to 12 random amino acids can be fused into pIII proteins to construct a library of approximately 10^8 different peptide sequences (see Figure 3). To select a suitable peptide sequence for binding specifically to a defined material, this phage library is exposed to the chosen material. Some of the phage in the library will bind to the chosen material, and some will not. The unbound phage are washed off, and the phage bound to the material are amplified into a sub-library and sequenced to identify the fused peptide sequences. This process is called biopanning and is repeated several times by exposing the sub-libraries to the chosen material until a consensus of peptide sequence is found. The sequence found in this biopanning process will possess specific affinity to the chosen material. This biopanning technique facilitates and speeds up the design process of peptides for binding materials.

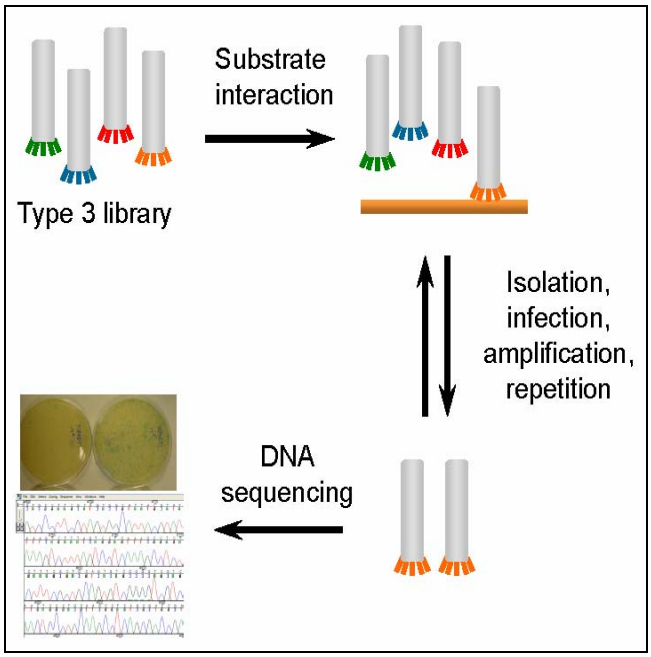

Figure 3. Biopanning of phage display library.

1.3 Biologically- based fibers

In addition to biomineralizaiton, nature has also taught us how to spin fibers with enormous strength and elasticity. Spiders and silk worms are known to use fibroin proteins in fabricating their perfect fibers through spinnerets. These spinnerets extrude liquid crystalline silk solutions into high strength and environmentally robust fibers. A number of groups have attempted to spin silk protein fibers, most involving *Bombyx* silk or regenerated spider silk (Vollrath, 2001; Koj,ic, 2004; and Um, 2004) . Several years ago, recombinant silk fibers were prepared from concentrated protein solutions by our group (Arcidiacono, 2002).

In nanoscience, bacteriophage templates have been used to make nanofilaments with functionalities as diverse as proteins. These nanofilaments form liquid crystal phases at high concentrations (Lee, 2002) and are therefore potential candidates for mimicking the process of silk fiber spinning. We hypothesized that fibers could be gernated from genetically controlled bacteriophage and used to mineralize metals and form metallic fibers. These biological materials could open a new synthesis route to manufacture multifunctional fibers. In this paper, we will

demonstrate the application of a genetically controlled filamentous bacteriophage in fabrication of functional fibers.

2. RESULTS

2.2 Bio-mineralization of M13 Bacteriophage

Due to the inherent structure of M13 bacteriophage, this filamentous virus is an excellent bio-template for the fabrication of metallic and semiconductive nano-filaments (or nano-wires), such as gold and cadmium sulfide. To genetically engineer a virus for manufacturing nano-filaments, biopanning is first performed as discussed above against a chosen material to find a suitable peptide motif. The Belcher lab has identified peptides specific for a number of materials such as GaAs, InP, GaN, FePt, CoPt, Ag, Au, ZnS, CdS, Si and many others. We have selected as a proof of principle a bacteriophage that has been genetically modified to express a gold binding peptide fused to the pVIII protein. Viral nano-filaments can be simply made out of the designed viral templates and precursor materials using sol-gel chemistry. This type of synthesis is performed at a mild condition, which provides an environmentally friendly synthesis route, and the results demonstrate the capabilities of the viral templates to control the morphologies of materials. Figure 4 shows an example of a gold nanowire of about 1 μm in length and 50 nm in width fabricated by an engineered M13 bacteriophage and gold precursors.

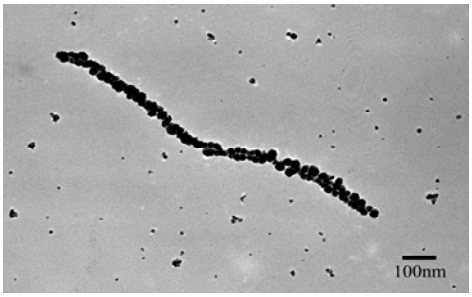

Figure 4. TEM images of viral gold nanowires

2.2 Fabrication of Viral Fibers

Synthetic polymers are commonly used as fiber materials; however, since it is difficult to produce varied functionalities on a single fiber surface, synthetic polymer-based fibers often lack functional versatility. Surface modifications of synthetic fibers usually require multistep chemical modification and/or expensive enzymes. Consequently, biologicals such as DNA, proteins, and microorganisms (e.g., virus and fungi) have become key materials design components due to their inherent controls of molecular functionalities and structures that they provide at the nanometer scale. I

To extend these applications, M13 filamentous bacteriophage were spun into continuous microfibers. These fibers can be made out of pure phage solution or a blended solution of phage and polymer to enhance the mechanical properties of viral fibers. When the virus clones are engineered to bind to inorganic materials and used to fabricate viral fibers, these fibers contain specifically designed functionality to form inorganic fibers at room temperature. We have synthesized gold fibers at room temperature from aqueous solutions using this genetic engineering and biomineralization process. Figure 5 depicts a schematic of the fiber spinning procedure.

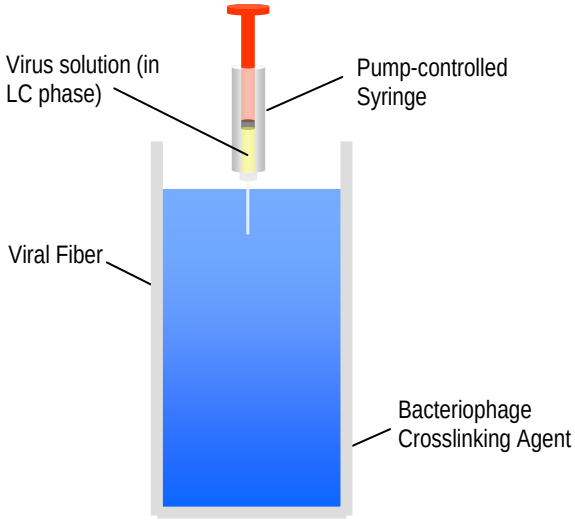

Figure 5. Schematic of phage fiber spinning process.

A continuous micro-fiber was created through a wet-spinning process where a concentrated virus solution (~ 470mg/ml in water, 1.7 x 10^{13} pfu/μL) was spun vertically through a 33 gauge needle into 2.5% glutaraldehyde solution at a constant rate, producing approximately 13 cm of viral fiber per μl of viral suspension. Fibers can be spun from both virus alone and QD-conjugated viral particles. After incubating in glutaraldehyde for 2 hours, viral fibers were rinsed with water. These fibers were then vertically pulled out by forceps and dried in air.

To test the gold mineralization capability of fibers, the wet fiber was immersed in a 300 μL chloroauric acid solution (5 mM, pH 7.5) and incubated on ice for 10 mins. A 150 μL sample of ice-cold sodium borohydride solution (5 mM) was then added to reduce the gold. After 12 hrs, the fiber was manually removed from the solution and rinsed with water. The fiber surface and the distribution of biotemplated gold on the viral fibers were analyzed using scanning electron microscopy (SEM) and energy dispersive X-ray imaging (EDX). The homogeneous coating of gold nanoparticles on the genetically

functionalized gold fibers indicates the correct presentation of functionalities on the virus fiber surface (see Figure 6).

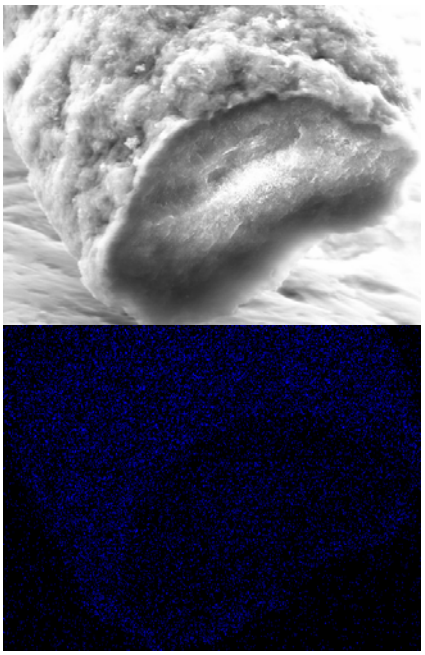

Figure 6. SEM and energy dispersive X-ray imaging of biomineralized gold fibers

In addition to reduction of gold on the surface of the fibers, we explored the binding of preformed gold nanoparticles. A continuous micro-fiber was produced using the same wet-spinning process described above. Fibers were drawn through a droplet of 10nm gold nanoparticles and rinsed with water. To demonstrate specificity of this gold-binding on genetically engineered M13 viral fibers, fibers were also spun from another clone, M13KE, which does not contain the gold-binding functionality.

Although crosslinking agents will inter-crosslink the amine groups at the N- termini of pVIII proteins, no disruption of surface functionality of the M13 viruses in these fibers was observed. This demonstrates the capability of the genetically engineered nanometric virus scaffold to mineralize inorganic materials and to retain the desired functionality at the micrometer scale.

Finally, figure 7 presents electron micrographs of the fibers following mechanical properties analysis. Macroscopically, the fracture mode of wet-spun viral fiber is brittle fracture; ductile fracture occurs locally inside the fibers. Young's modulus was determined to be 1~4 GPa (Kevlar: 44-49GPa; Polystyrene: 2-4 GPa; Nylon 66: 1-3GPa).

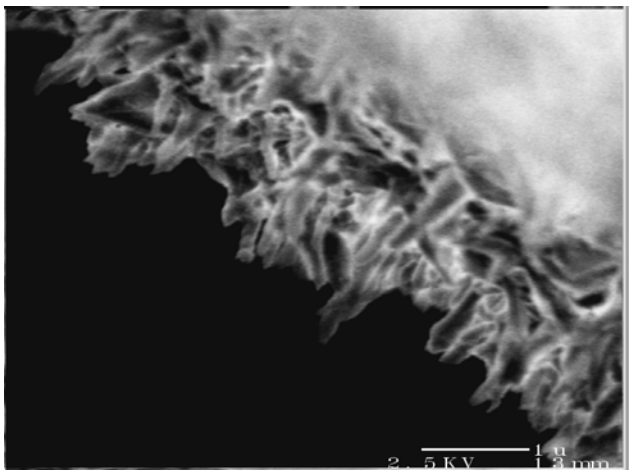

Figure 7. SEM image of fractured phage fibers.

3. CONCLUSION

The M13 filamentous viruses are self-amplified in bacteria hosts, which gives low material cost for scale-up commercialization. M13 virus-based fibers have great potentials of being used as functional fibers in a variety of applications, such as electronics, optics, tissue scaffold, protective textile, catalysis and filtration. The surface functions of these viral fibers can be easily altered and designed for diverse uses through genetic engineering techniques.

The functionalities, thermal and mechanical properties of the viral fibers show the promise that these high aspect ratio structures may be useful materials for various applications such as catalysis, energy storage, and energy generation In addition to simply binding to inorganic materials, viral fibers also can be designed to bind organic dyes, biomolecules, toxic gases, etc, by functionalizing individual viruses.

We demonstrate the potential of this virus to serve as a powerful toolkit for designing a specific functional fibrous material from standard biotechniques including biopanning, bacteriophage amplification, and genome modification. The viral fibers possess mechanical toughness, strength, and thermal stability comparable to synthetic polymer fibers, indicating that this filamentous virus can be integrated into current fibril manufacturing systems. The genetic manipulation of diverse functionalities on the viral fibers offers a convenient and powerful basis for conjugating organic or inorganic materials for a variety of applications such as the creation of anti-microbial, catalytic, optical, medical and electronic materials.

The availability of functionalized fibers that can be spun or assembled at high concentrations, and in which the

fibers themselves have structural integrity, provide the potential for sensors that could be integrated into a uniform to enable early warning of battlefield hazards. Other possible uses include use in flexible displays, friend vs. foe ID, electronic and smart textiles for use in airdrop and flexible structures.

4. REFERENCES

Arcidiacono, S., Mello, C.M., Butler, M., Welsh, E., Soares, J.W., Allen, A., Ziegler, D., Laue, T., and Chase, S., 2002: Aqueous processing and fiber spinning of recombinant spider silks, *Macromol.* 35: 1262-1266.

Dickerson, M. B., Naik, R. R., Stone, M. O., Cai, Y., and Sandhage, K. H., 2004: Identification of peptides that promote the rapid precipitation of Germania nanoparticle networks via use of a peptide display library, *Chem. Commun.* 1776: 1776-1777.

Flynn, C. E., Lee, S. W., Peelle, B. R. & Belcher, A. M. 2003: Viruses as vehicles for growth, organization and assembly of materials, *Acta Materialia* 51, 5867-5880.

Goede, K., Busch, P., and Grundmann, M., 2004: Binding Specificity of a Peptide on Semiconductor Surfaces, *Nano Letters* 4: 2115-2120.

Kojic, N., Kojic, M., Gudlavalleti, S., McKinley, G., 2004: Solvent removal during synthetic and *Nephila* fiber spinning, *Biomacro.* 5: 1698-1707.

Lee, S-W., Mao, C., Flynn, C. E., Belcher, A. M., 2002: Ordering of quantum dots using genetically engineered viruses, *Science* 296: 892.

Mao, C., et al., 2004: Virus-based toolkit for the directed synthesis of magnetic and semiconducting nanowires, *Science* 303, 213-217.

Nam, K. T., et al, 2006: Virus-enabled synthesis and assembly of nanowires for lithium ion battery electrodes. *Science* 312, 885-888.

Ogasawara, W., Shenton, W., Davis, S.A., and Mann, S. 2000: Template mineralization of ordered macroporous chitin-silica composites using cullebone-derived organic matrix, *Chem. Mater.* 12: 2835-2837.

Oren, E.E., Tamerler, C., and Sarikaya, M., 2005: Metal recognition of septapeptides via polypod molecular architecture, *Nano Letters* 5: 415-419.

Um, I.C., Kweon, H.Y., Lee, K. G., Ihn, D.W., Lee, J-H., Park, Y. H. 2004: Wet spinning of silk polymer I. Effect of coagulation conditions on the morphological feature of filament, *Int J Biol. Macromol.* 34: 89-105.

Young, J.R., Didymus J. M. Bown P. R. Prins B. and Mann, S., 1992: Crystal assembly nad phylogenetic evolution in heterococcoliths, *Nature* 365: 516.

Volrath, F., and Knight, D.P., 2001: Liquid crystalline spinning of spider silk, *Nature* 410: 541.